Human / Robotic Hematopoietic Stem Cell Therapy and Gene Therapy for Exploration of the Solar System

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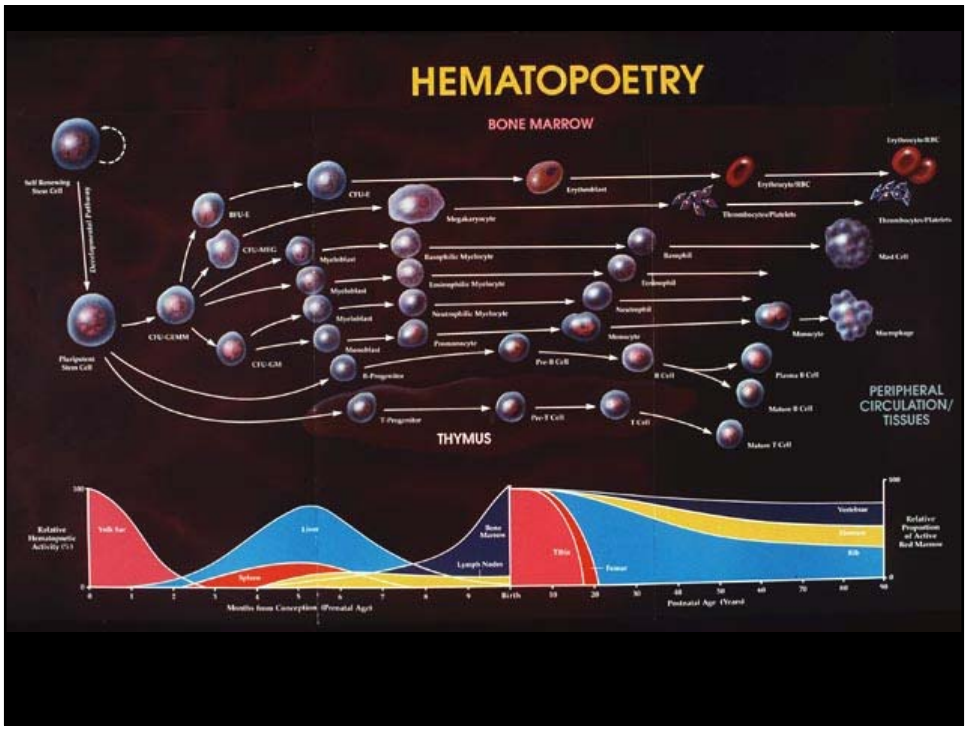
Interplanetary Space Exploration:

A) Readiness for catastrophic disorders:
- Cancer
- Immunodeficiency
- Muscle and bone losses
- Hematological and cardiac abnormalities
- Neurological disorders

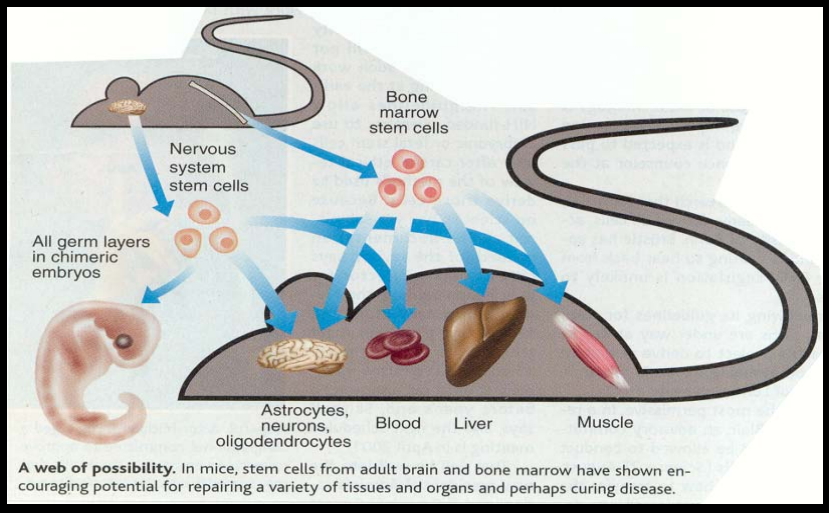
B) Countermeasures:
- Hematopoietic stem cell therapy (HSCT)
- Gene therapy
- Others

C) Robotization; Robotic medicine

HEMATOPOIETIC STEM CELL THERAPY (HSCT)



Multiple Differentiating Potential of Adult Stem Cells



A web of possibility. In mice, stem cells from adult brain and bone marrow have shown encouraging potential for repairing a variety of tissues and organs and perhaps curing disease.

Adapted from: Vogel G, *Science* **290**:1674 (2000)

Possible Benefits of HSCT

- Cancer Therapy:
  - Bone marrow replacement therapy for blood-born cancer, e.g. leukemia
  - High dose chemotherapy
- Hematological disorders:
  - Space anemia
  - Immunodeficiency
- Muscle and bone losses
- Neurological disorders

PURIFICATION OF MOUSE HSCs

Flusihning Tibiae and Femora

↓

Bone Marrow Cells

↓

Density Gradient Centrifugation in Histopaque 1077

↓

Interface Cells

↓

Negative Selection

(T cells were identified by rat anti-mouse Lyt-2 Ab + anti-mouse L3T4 Ab, and removed by magnetic goat anti-rat IgG Ab on the BioMag Separator)

↓

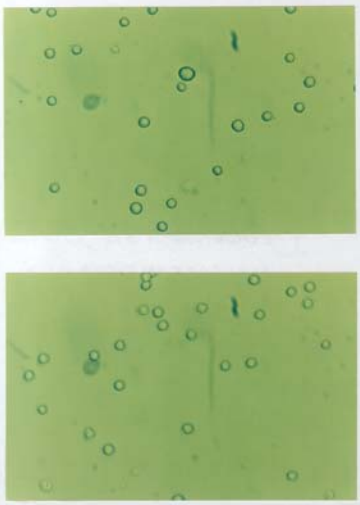
Positive Selection

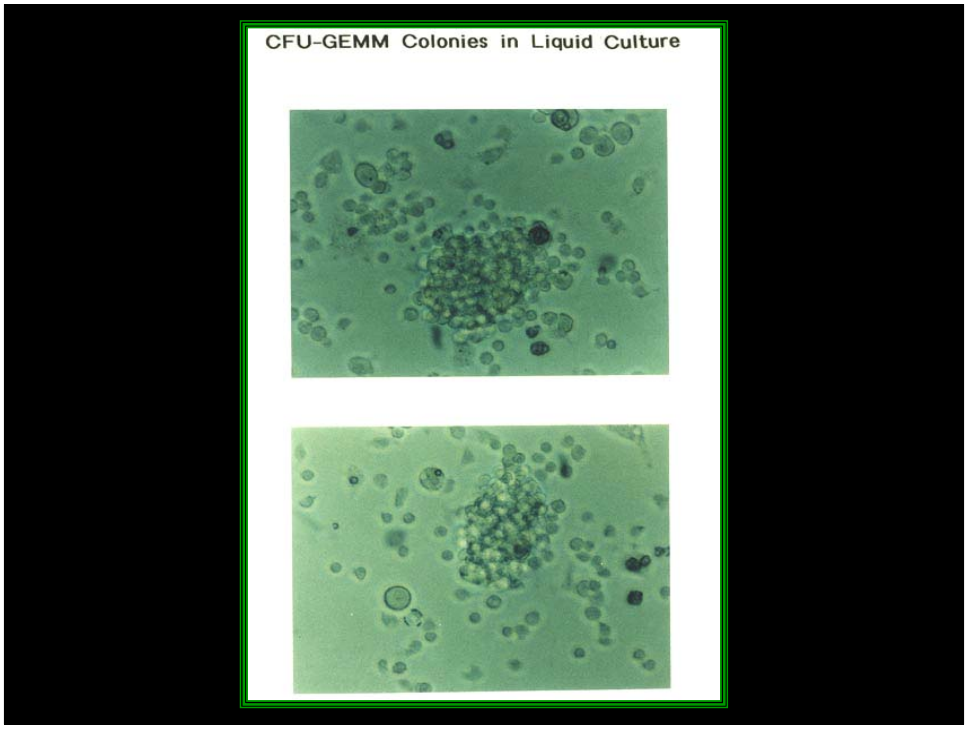
(HSCs were identified by first, rat anti-mouse Thy-1.2 Ab and second, rat anti-Sca-1 Ab, and selected each step by magnetic goat anti-rat IgG Ab on the BioMag Separator)

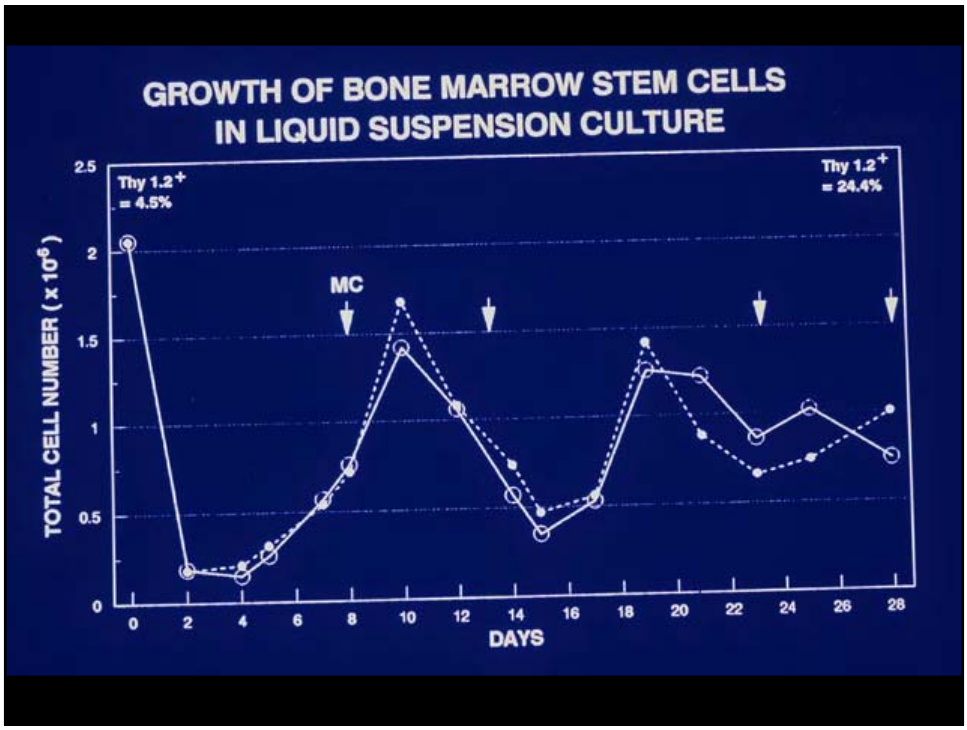
↓

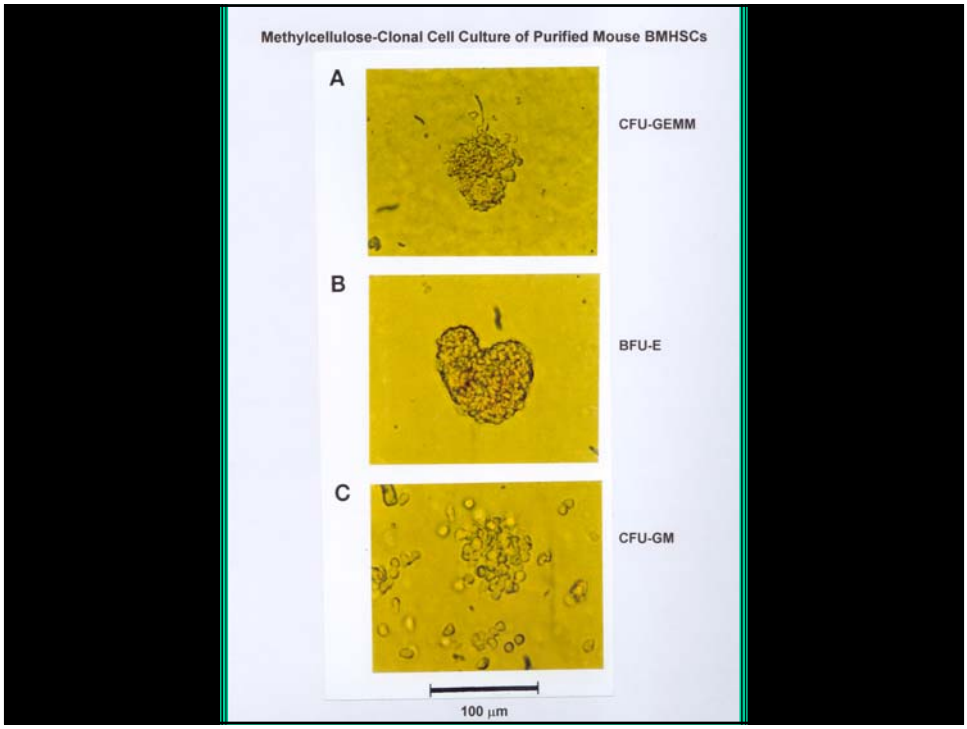
HSCs

Purified Mouse BMHSCs



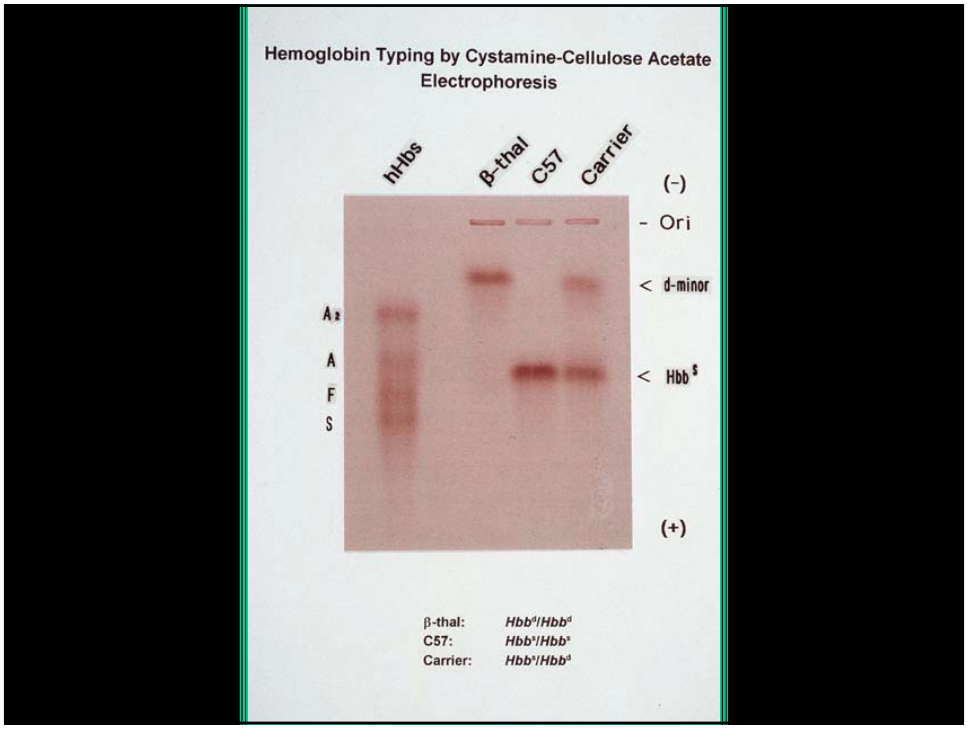

CFU-GEMM Colonies in Liquid Culture

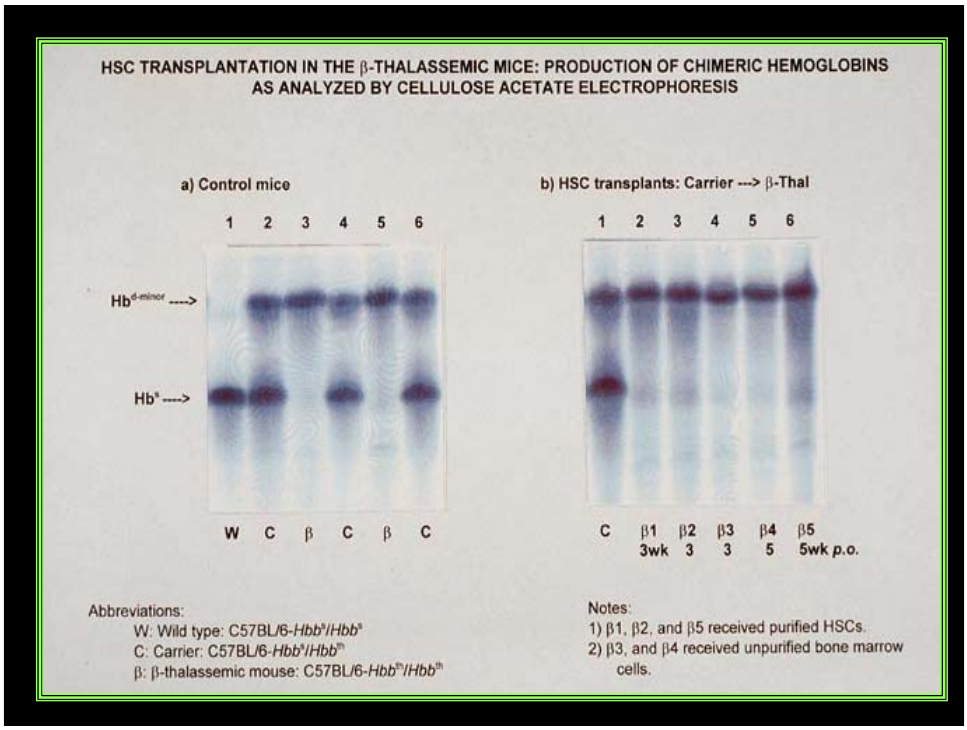

GROWTH OF BONE MARROW STEM CELLS
IN LIQUID SUSPENSION CULTURE
Thy 1.2+
= 4.5%
Thy 1.2+
= 24.4%
MC
TOTAL CELL NUMBER (x 10^6)
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
DAYS



The β-Thalassemic Mouse

WT β-THAL


Hemoglobin Typing by Cystamine-Cellulose Acetate Electrophoresis
hHbs
β-thal
C57
Carrier
(-)
- Ori
< d-minor
< Hbb$
A2
A
F
S
(+)
β-thal: Hbb^d/Hbb^d
C57: Hbb^s/Hbb^s
Carrier: Hbb^s/Hbb^d


HSC TRANSPLANTATION IN THE β-THALASSEMIC MICE: PRODUCTION OF CHIMERIC HEMOGLOBINS AS ANALYZED BY CELLULOSE ACETATE ELECTROPHORESIS
a) Control mice
1 2 3 4 5 6
Hb^d-minor ---->
Hb^s ---->
W C β C β C
b) HSC transplants: Carrier ---> β-Thal
1 2 3 4 5 6
C β1 β2 β3 β4 β5
3wk 3 3 5 5wk p.o.
Abbreviations:
W: Wild type: C57BL/6-Hbb^s/Hbb^s
C: Carrier: C57BL/6-Hbb^s/Hbb^th
β: β-thalassemic mouse: C57BL/6-Hbb^th/Hbb^th
Notes:
1) β1, β2, and β5 received purified HSCs.
2) β3, and β4 received unpurified bone marrow cells.

GENE THERAPY

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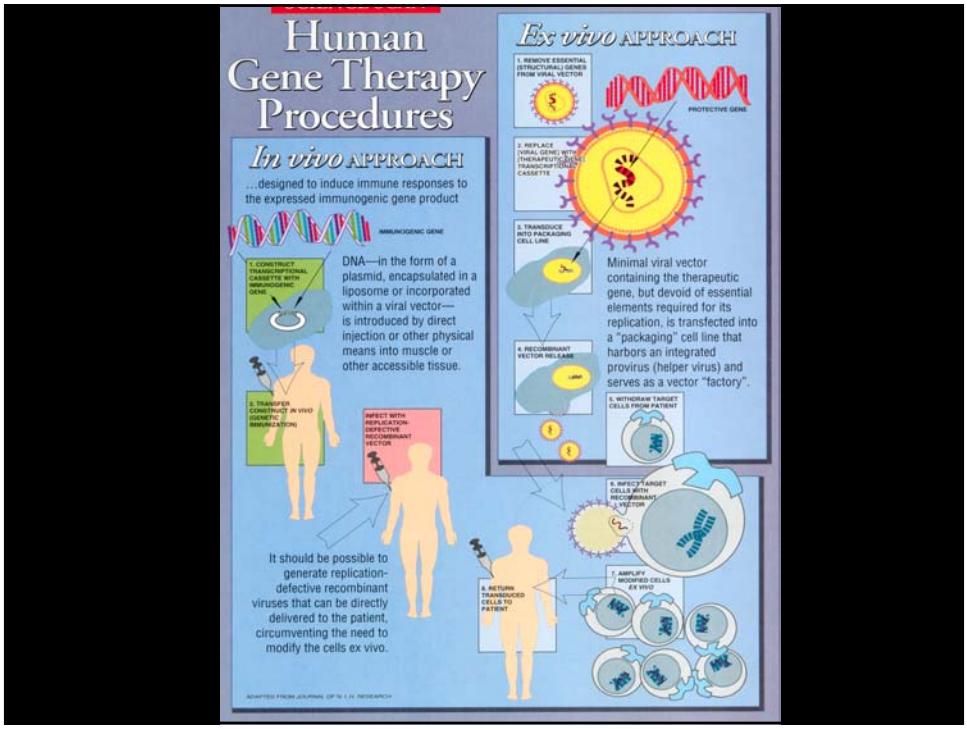
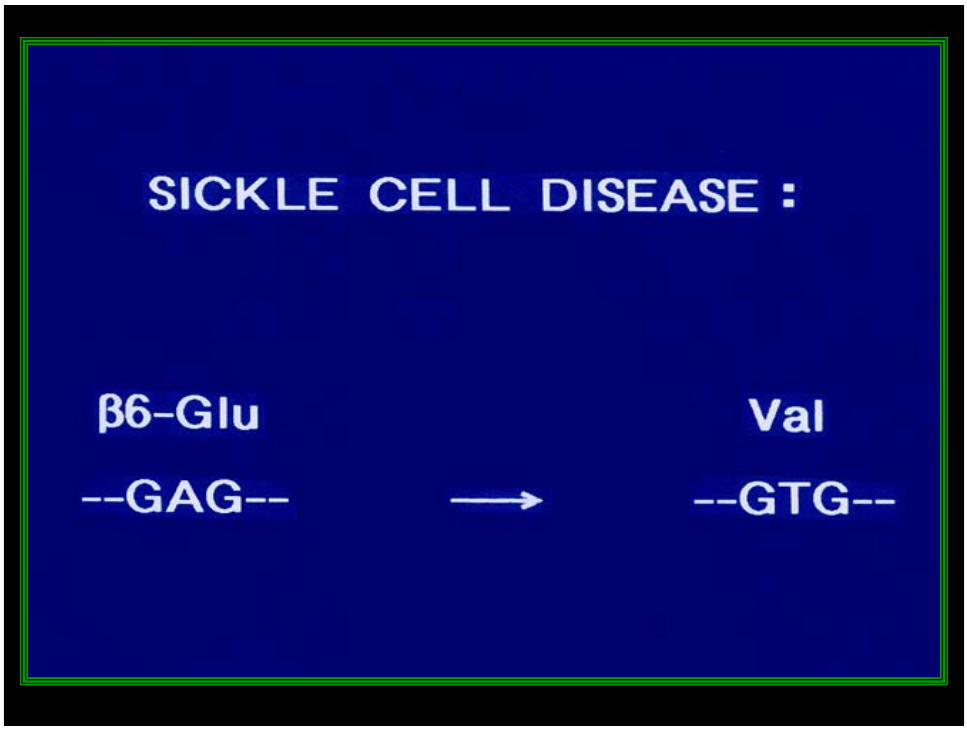
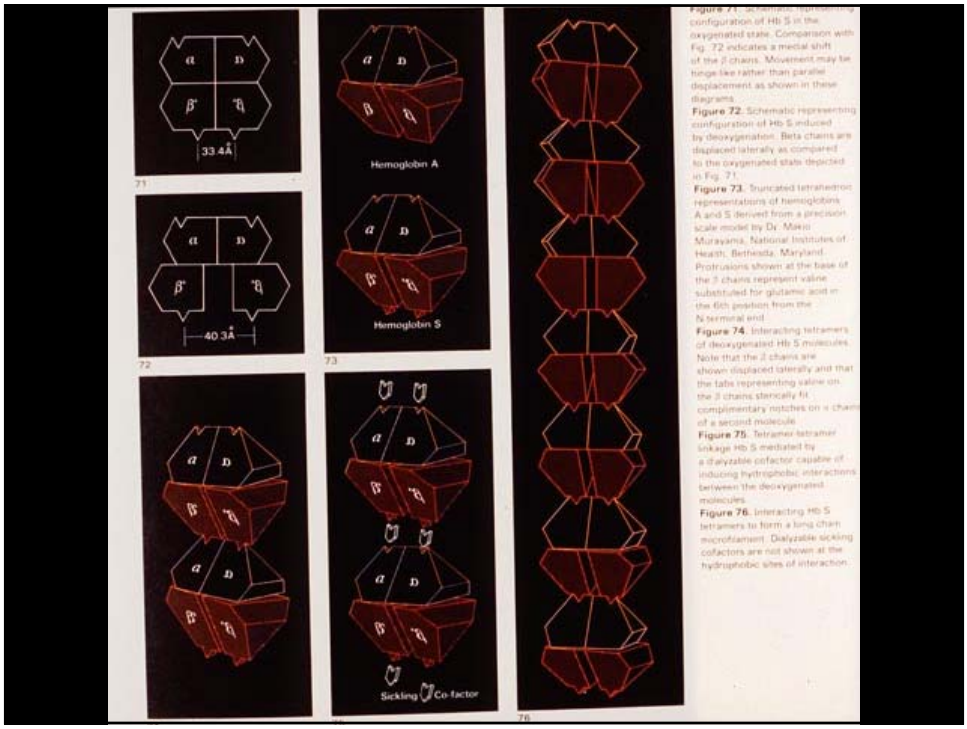
FOR

1. GENETIC DISEASES
   e.g. Sickle Cell Disease, β-Thalassemia

2. ACQUIRED DISEASES
   e.g. Cancer, AIDS, Space Anemia


Human Gene Therapy Procedures
In vivo APPROACH
...designed to induce immune responses to the expressed immunogenic gene product
IMMUNOGENIC GENE
1. CONSTRUCT TRANSCRIPTIONAL CASSETTE WITH IMMUNOGENIC GENE
DNA—in the form of a plasmid, encapsulated in a liposome or incorporated within a viral vector—is introduced by direct injection or other physical means into muscle or other accessible tissue.
2. TRANSFER CONSTRUCT IN VIVO (GENETIC IMMUNIZATION)
INFECT WITH REPLICATION-DEFECTIVE RECOMBINANT VECTOR
It should be possible to generate replication-defective recombinant viruses that can be directly delivered to the patient, circumventing the need to modify the cells ex vivo.
Ex vivo APPROACH
1. REMOVE ESSENTIAL (STRUCTURAL) GENES FROM VIRAL VECTOR
PROTECTIVE GENE
2. REPLACE (VIRAL GENE) WITH (THERAPEUTIC GENE) TRANSCRIPTIONAL CASSETTE
3. TRANSDUCE INTO PACKAGING CELL LINE
Minimal viral vector containing the therapeutic gene, but devoid of essential elements required for its replication, is transfected into a "packaging" cell line that harbors an integrated provirus (helper virus) and serves as a vector "factory".
4. RECOMBINANT VECTOR RELEASE
5. WITHDRAW TARGET CELLS FROM PATIENT
6. INFECT TARGET CELLS WITH RECOMBINANT VECTOR
7. AMPLIFY MODIFIED CELLS EX VIVO
8. RETURN TRANSDUCED CELLS TO PATIENT


SICKLE CELL DISEASE :
β6-Glu
Val
--GAG--
--GTG--



configuration of Hb S in the oxygenated state. Comparison with Fig. 72 indicates a medial shift of the β chains. Movement may be hinge-like rather than parallel displacement as shown in these diagrams.

Figure 72. Schematic representing configuration of Hb S induced by deoxygenation. Beta chains are displaced laterally as compared to the oxygenated state depicted in Fig. 71.

Figure 73. Truncated tetrahedron representations of hemoglobins A and S derived from a precision scale model by Dr. Makio Murayama, National Institutes of Health, Bethesda, Maryland. Protrusions shown at the base of the β chains represent valine substituted for glutamic acid in the 6th position from the N-terminal end.

Figure 74. Interacting tetramers of deoxygenated Hb S molecules. Note that the β chains are shown displaced laterally and that the tabs representing valine on the β chains sterically fit complementary notches on α chains of a second molecule.

Figure 75. Tetramer-tetramer linkage Hb S mediated by a dialyzable cofactor capable of inducing hydrophobic interactions between the deoxygenated molecules.

Figure 76. Interacting Hb S tetramers to form a long chain microfilament. Dialyzable sickling cofactors are not shown at the hydrophobic sites of interaction.

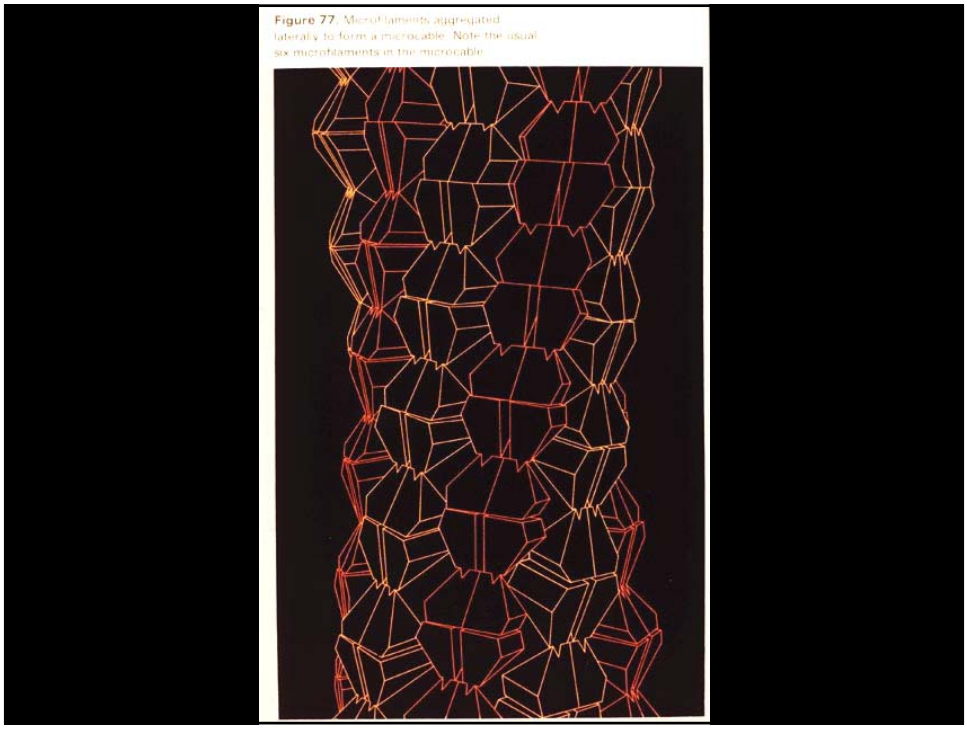


Figure 77. Microfilaments aggregated laterally to form a microcable. Note the usual six microfilaments in the microcable.

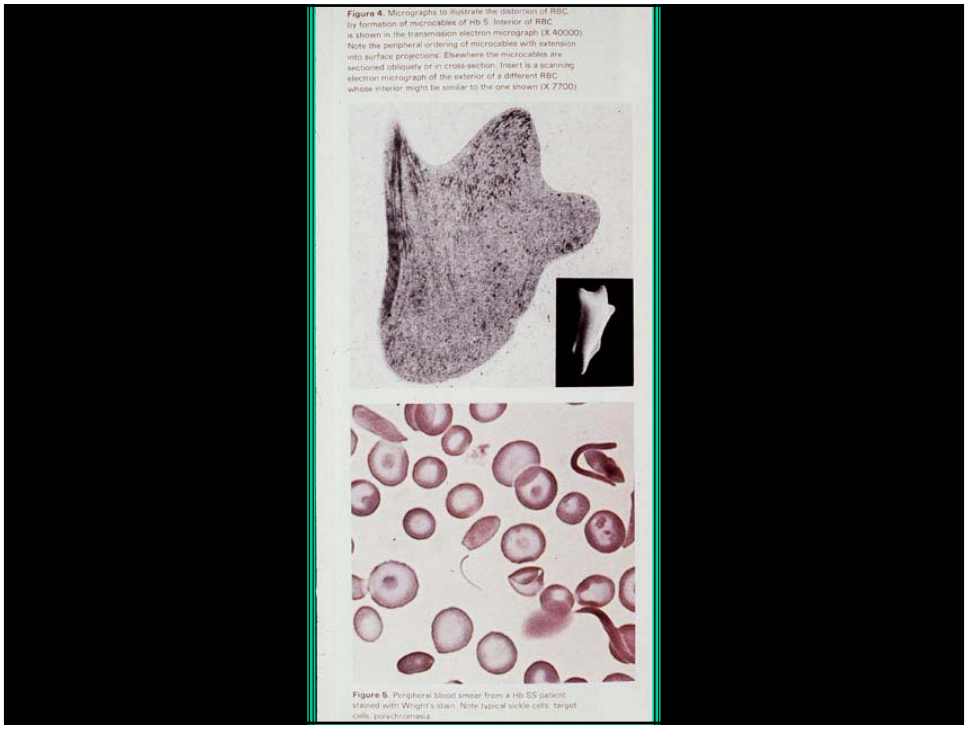


Figure 4. Micrographs to illustrate the distortion of RBC by formation of microcables of Hb S. Interior of RBC is shown in the transmission electron micrograph (X 40000). Note the peripheral ordering of microcables with extension into surface projections. Elsewhere the microcables are sectioned obliquely or in cross-section. Insert is a scanning electron micrograph of the exterior of a different RBC whose interior might be similar to the one shown (X 7700).

Figure 5. Peripheral blood smear from a Hb SS patient stained with Wright's stain. Note typical sickle cells, target cells, polychromasia.

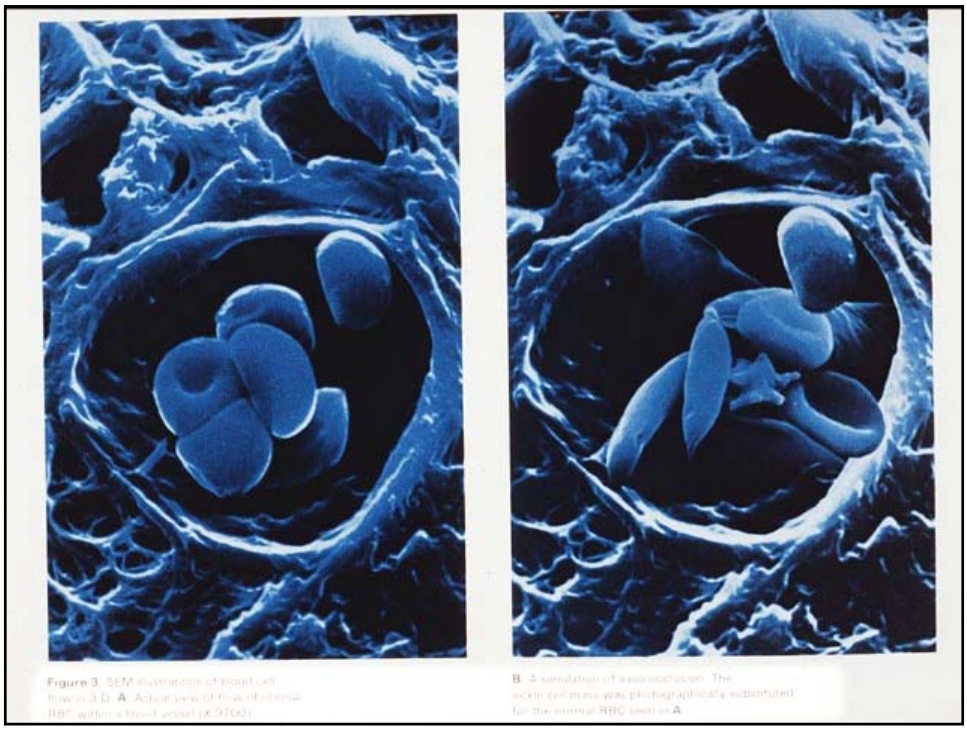
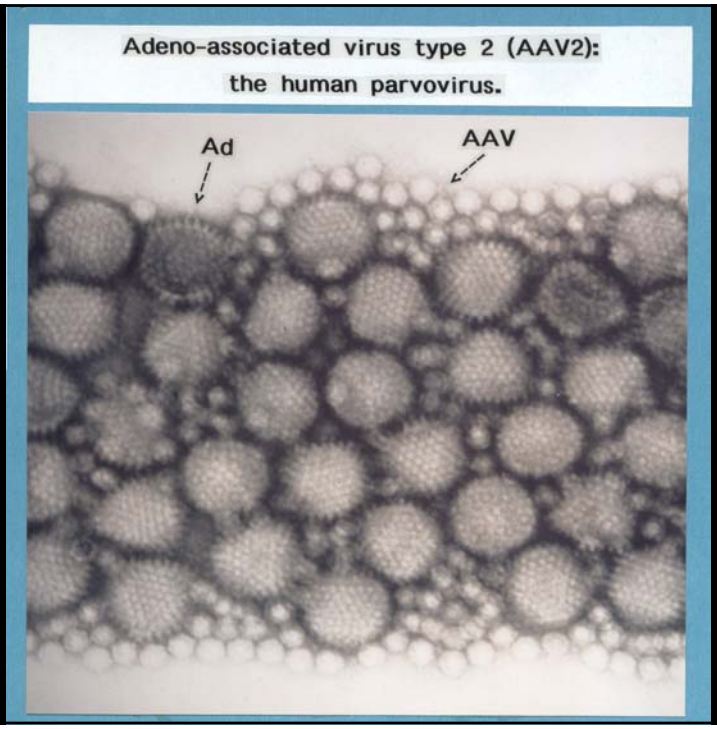


Figure 3. SEM illustrations of blood cell flow in 3-D. **A.** Actual view of flow of normal RBC within a blood vessel (X 9700).

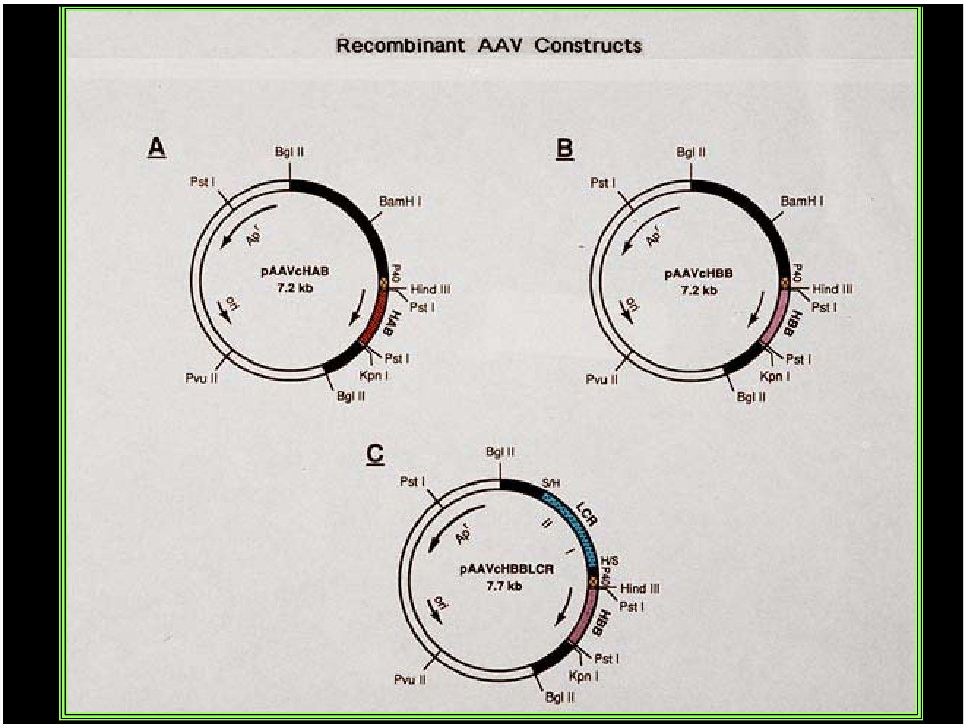
B. A simulation of sago occlusion. The sickle cell mass was photographically substituted for the normal RBC seen in **A**.

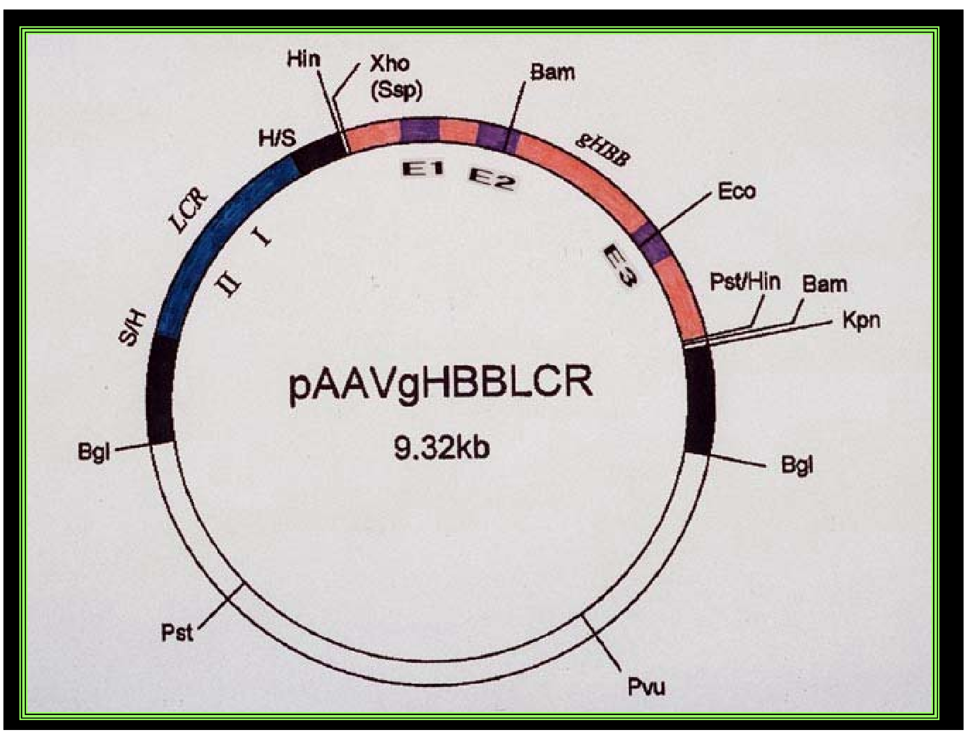
OUR AIM IS TO DEVELOP RECOMBINANT ADENO-ASSOCIATED VIRUSES THAT CONTAIN HUMAN GLOBIN GENES FOR GENE THERAPY OF HEMOGLOBINOPATHIES

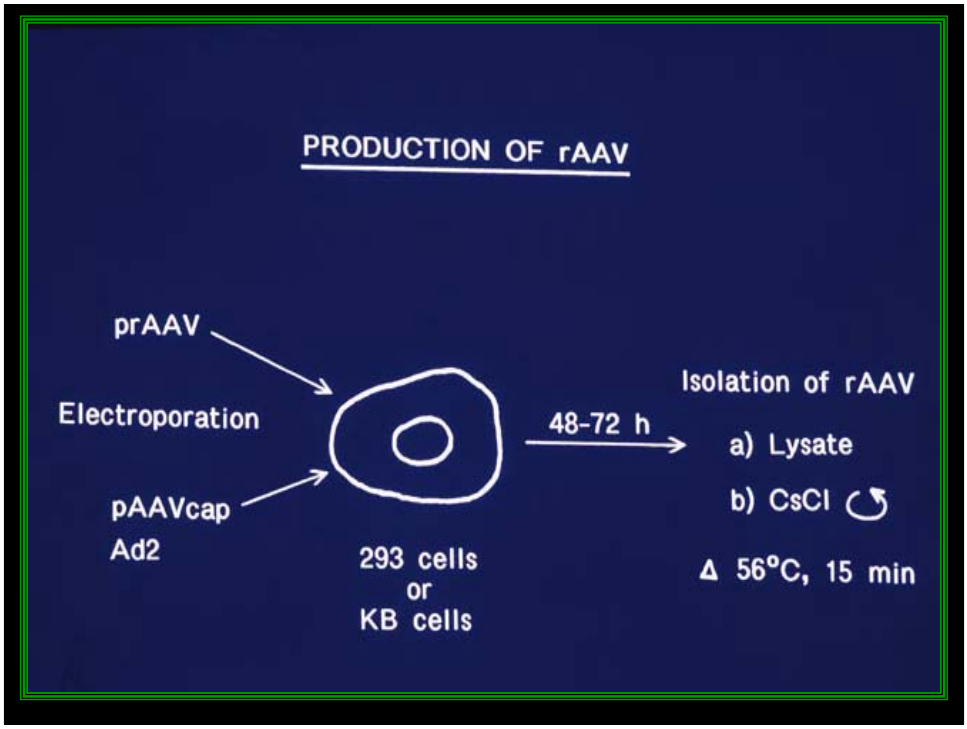


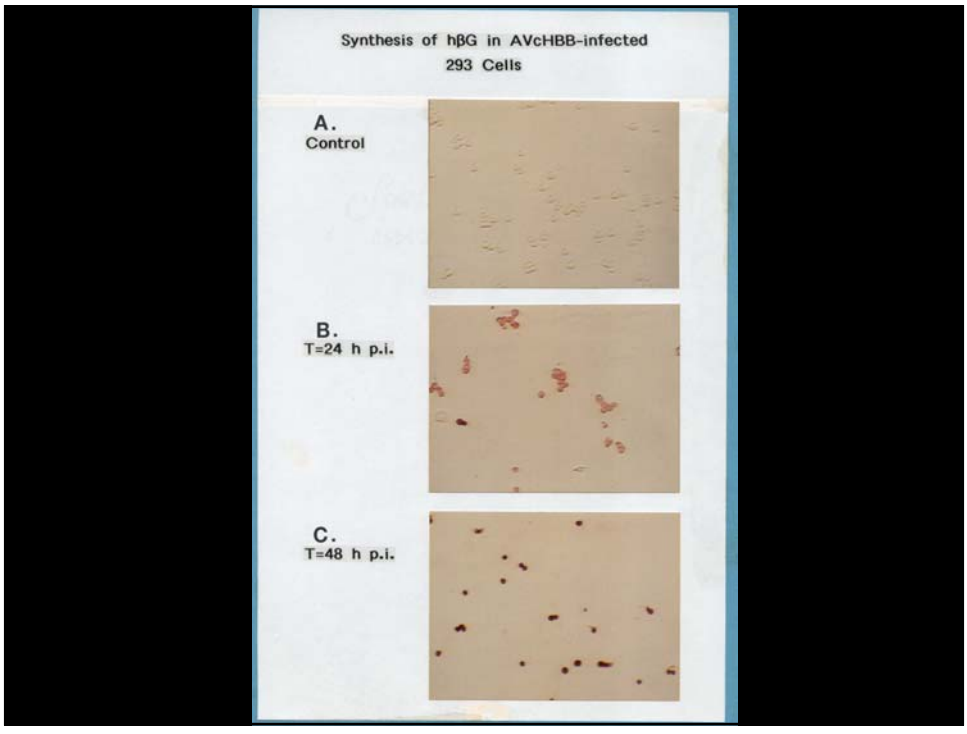
POSSIBLE ADVANTAGES OF AAV SYSTEM OVER RETROVIRUS SYSTEM IN GENE THERAPY

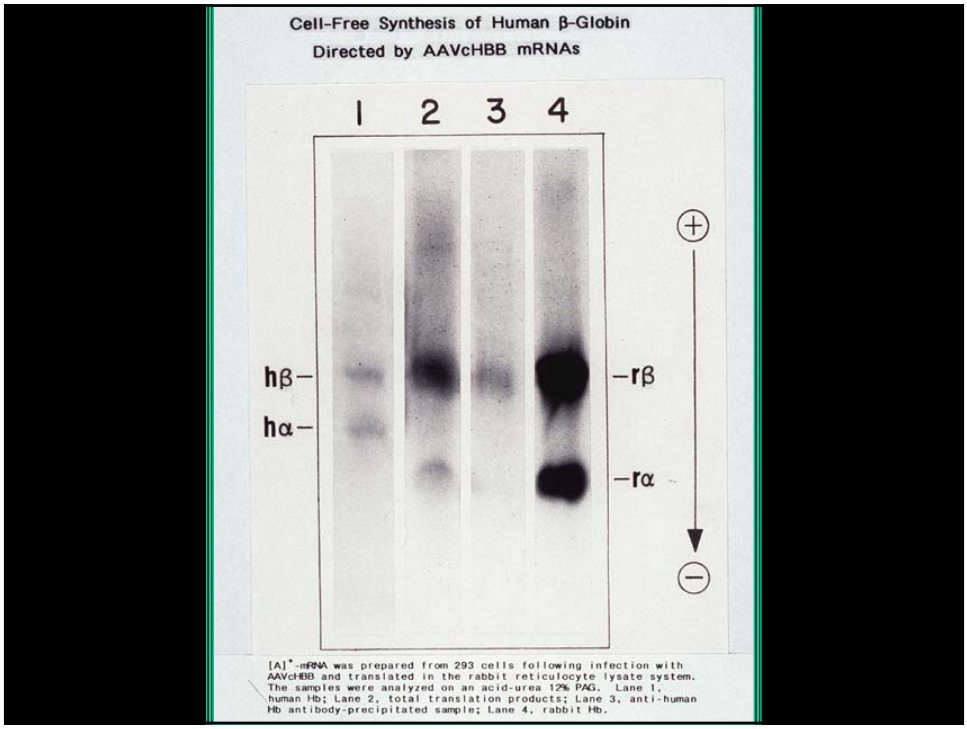
1. AAV is a non-pathogen: gene therapy by *in vivo* infection.
2. Two modes of infection:
   a) Lytic infection: transient expression
   b) Integrative infection: proviral expression.
3. Hardy virus:
   a) 60°C, 30 min.
   b) Purification by CsCl density gradient: high titer stock.
4. Broad host-range and tissue specificity.
5. AAV infects non-dividing cells. Expression?
6. Gene therapy *via* nucleated cells (eg. nucleated RBC).

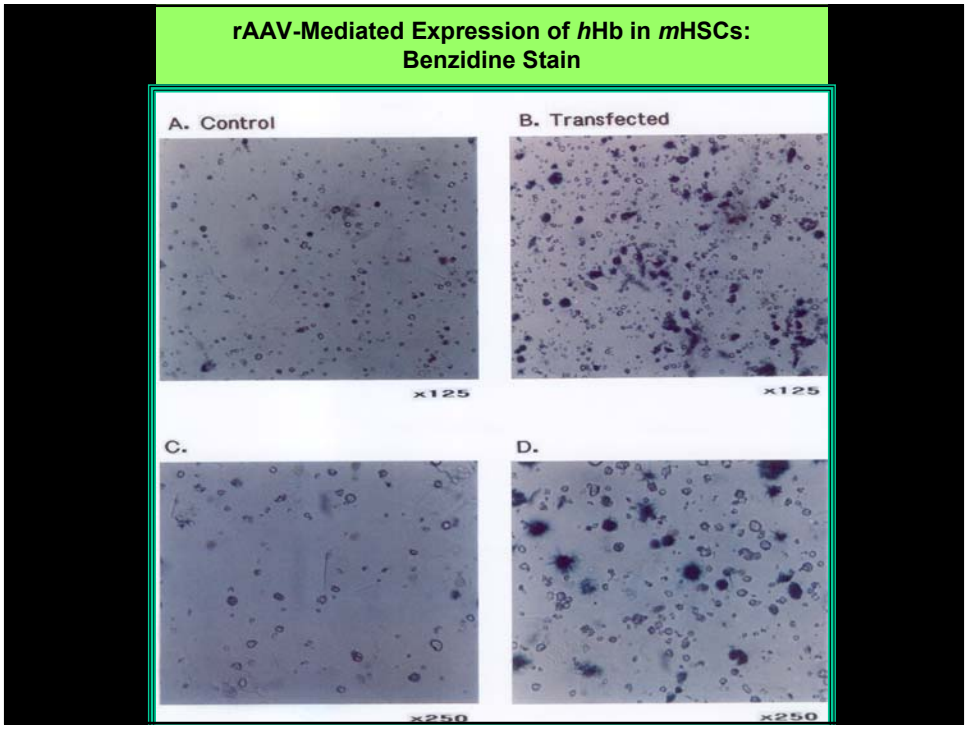

Recombinant AAV Constructs
A
Bgl II
Pst I
BamH I
Apr
pAAVcHAB
7.2 kb
P40
Hind III
Pst I
ori
HAB
Pst I
Kpn I
Pvu II
Bgl II
B
Bgl II
Pst I
BamH I
Apr
pAAVcHBB
7.2 kb
P40
Hind III
Pst I
ori
HBB
Pst I
Kpn I
Pvu II
Bgl II
C
Bgl II
Pst I
S/H
LCR
II
I
Apr
H/S
P40
pAAVcHBBLCR
7.7 kb
Hind III
Pst I
ori
HBB
Pst I
Kpn I
Pvu II
Bgl II

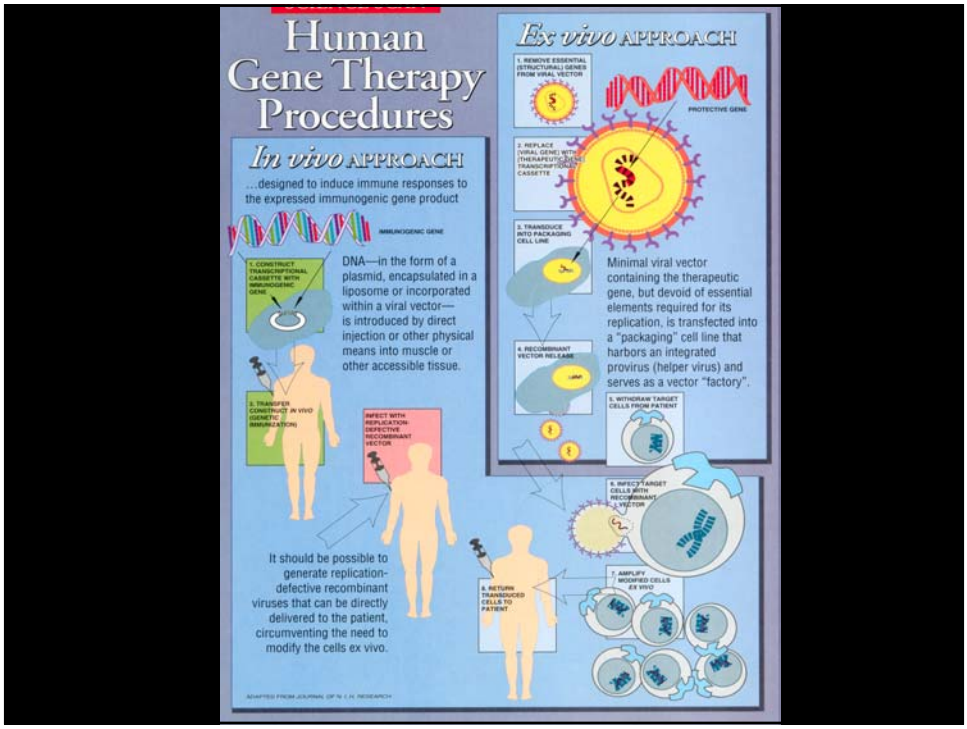

Hin
Xho
(Ssp)
Bam
H/S
gHBB
E1
E2
LCR
I
II
Eco
E3
Pst/Hin
Bam
Kpn
S/H
pAAVgHBBLCR
9.32kb
Bgl
Bgl
Pst
Pvu


PRODUCTION OF rAAV
prAAV
Electroporation
pAAVcap
Ad2
293 cells
or
KB cells
48-72 h
Isolation of rAAV
a) Lysate
b) CsCl
Δ 56°C, 15 min


Synthesis of hβG in AVcHBB-infected
293 Cells
A.
Control
B.
T=24 h p.i.
C.
T=48 h p.i.


Cell-Free Synthesis of Human β-Globin
Directed by AAVcHBB mRNAs
1 2 3 4
hβ–
hα–
–rβ
–rα
⊕
⊖
[A]+-mRNA was prepared from 293 cells following infection with AAVcHBB and translated in the rabbit reticulocyte lysate system. The samples were analyzed on an acid-urea 12% PAG. Lane 1, human Hb; Lane 2, total translation products; Lane 3, anti-human Hb antibody-precipitated sample; Lane 4, rabbit Hb.

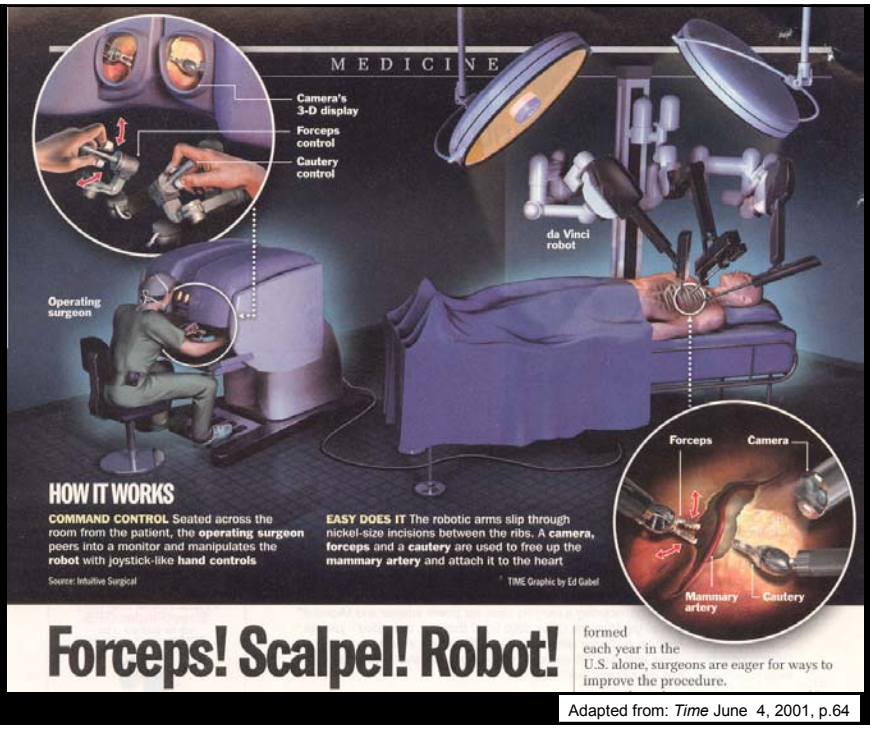

rAAV-Mediated Expression of *h*Hb in *m*HSCs:
Benzidine Stain
A. Control
B. Transfected
x125
x125
C.
D.
x250
x250



Possible Benefits of Gene Therapy

- Genetic engineering of astronauts for radiation resistance and other disorders
- Cancer therapy
  - Tumor suppressor genes
  - Multidrug resistant gene for high dose chemotherapy
- Hematological disorders
  - Low hemoglobin concentration (MCHC)
  - Erythropoietin production
- Muscle and bone losses
  - Growth hormones

ROBOTIC MEDICINE



Adapted from: *Time* June 4, 2001, p.64

PREVENTIVE MEDICINE based on human / robotic synergistic diagnosis and minor operations:

Hematopoietic Stem Cell Therapy and Gene Therapy

HUMAN/ROBOTIC HSCT AND GENE THERAPY FOR EXPLORATION OF THE SOLAR SYSTEM

SYNOPSIS

1. Long-duration space mission requires countermeasures against catastrophic disorders: cancer, bone & muscle losses, etc.
2. HSCT and GT could provide countermeasures/cures to these disorders.
3. Methods for purification and long-term culture of HSCs are established; research on AAV-mediated gene therapy is underway: *Ohi et al.*
4. To enable these treatments by crewmembers in space, the following procedures need to be established and automated/robotized:

- Robotic diagnosis of health status and prescription.
- Engineering of robotic HSC purification machine.
- Automated device for growing HSCs in low/0 G from the frozen state: Rf. Todd et al.

HUMAN/ROBOTIC HSCT AND GENE THERAPY FOR EXPLORATION OF THE SOLAR SYSTEM

SYNOPSIS-continued

- HSC delivery mechanisms:
  - a) Gastric resistant capsule form; automated packaging of HSCs in capsule.
  - b) Intravenous injection: development of *i.v.* injection machine.
- HSC therapy for muscle and bone losses: *Ohi & Shapiro*
  - a) Targeting of HSCs to the tissues.
  - b) Study of the effect of HSC therapy on muscle and bone losses, using rodent hind-limb suspension model.
- Establishment of *ex vivo* gene therapy protocols in space using a mouse model of β-thalassemia: *Ohi, Margolis, Fitsgerald et al.*
- Human clinical trials for HSC therapy and gene therapy in space.